aHUS: Facts, Controversies & Treatment Updates

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DISCLOSURE STATEMENT

I, **Patrick Brophy** disclose the following relationships. Any real or apparent conflicts of interest related to the content of this presentation have been resolved.

Affiliation / Financial Interest	Organization
Advisor	Alexion – International Atypical Hemolytic Uremic Syndrome Advisory Board

The following presentation will not discuss unapproved or off-label, experimental or investigational use of medications.

Objectives

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- Review the diagnosis of aHUS
  - What its is
  - Genetics behind it
- Case presentations
  - Diagnostic dilemmas
- Treatment option
  - Old & New
- Homegrown perspectives

aHUS FACTS

aHUS?

TTP

HIT

AMR

aHUS

STEC HUS

AKI

DIC

TMA

ITP

HELLP

MAHA

SIRS

AI

A Classification of TMA

(Thrombotic Microangiopathy)

Typical / diarrheal	**(HUS or TTP)**
Complement defects	**Atypical HUS**
von Willebrand proteinase (ADAMSTS13) defeciency	**Generally TTP**
Cobalamin-C deficiency	**TMA + multiorgan failure**
Quinine-related	**Abrupt TMA, exposure related**
Post transplantation (calcineurin inhibitior related)	**De-novo renal TMA May be renal “isolated”**
Others: HIV, radiation, chemotherapy HELLP, antiphospholipid Ab syndrome, unclassified	

Besbas et al. Kidney International 2006

Typical HUS

Triad of :

- **Microangiopathic hemolytic anemia**
- **Thrombocytopenia**
- **Acute kidney injury**

Generally <u>diarrhea-associated</u>

- **Shiga toxin produced by *E coli* serotype O157:H7**
- **Shigella, Salmonella, others also**
- **Food borne disease: uncooked / unpasteurized products contaminated by animal wastes**

Or other infections (respiratory):

- **Invasive *S. Pneumoniae* or viral infections**

Typical HUS

A severe condition:

acutely 2.5% mortality, often significant morbidity

Long term results (10-20 years after HUS*)

63% Complete recovery

12% Recovery with proteinuria

6% Recovery with proteinuria and HTN

16% Recovery with low GFR ± proteinuria or HTN

3% ESRD

**** Diarrheal or URI- related only, pediatric***

Spizzirri et al. Pediatric Nephrology 1996

Atypical HUS

Clinically very severe

15% died
25% ESRD **60% major sequelae**
15% renal insufficiency

1/3 recover without significant renal disease
most (75%) of these had a single episode
few (25%) of these had recurrent aHUS

(a pediatric series)

Taylor et al Ped Neph 2004

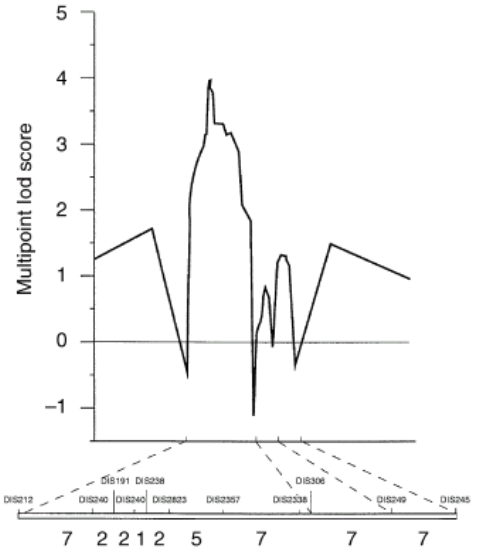
Laboratory evaluation of suspected aHUS	
R/O STEC HUS	Stool or rectal swab: culture for STEC; PCR for Stx Serum: Antibody testing
R/O Streptococcus pneumoniae infection	Blood cultures, pulmonary cultures
R/O Thrombotic thrombocytopenia purpura	Plasma ADAMTS13 activity ± inhibitor
R/O Other secondary causes of TMA	Blood pressure, pancreatitis, malignancy, medication
R/O Cobalamin metabolism abnormality (i.e. methyl-malonic aciduria))	Homocysteine, Methionine, urine organic acid testing Consider genetic testing for mutation in MMACHC gene
R/O Rheumatologic Disease	Antinuclear antibody, lupus anticoagulant, anti-phospholipid antibodies
R/O HIV	HIV Serology
R/O Pregnancy Associated TMA/HELLP Syndrome	Pregnancy test, liver enzymes.
Rule in Complement Pathway Abnormality	C3, Factor H, Factor I, Factor B serology Anti-factor H autoantibodies MCP - surface expression on leucocytes by FACS Gene mutation analysis for CFH, CFI, CFB, C3, CFHR5, MCP and CFHR1/3 Deletion

Complement and Atypical HUS

Since the early 1970's alternative pathway complement activation (low C3), has been recognized in some cases of atypical HUS

1981: 1st case of HUS with factor H deficiency described

1998: Linkage analysis in 3 families with HUS provided clear association with *CFH*



Clin. Exp. Immunol. (1981), Kidney International (1998)

Atypical HUS

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COMPLEMENT DYSREGULATION

- complement components and pathways

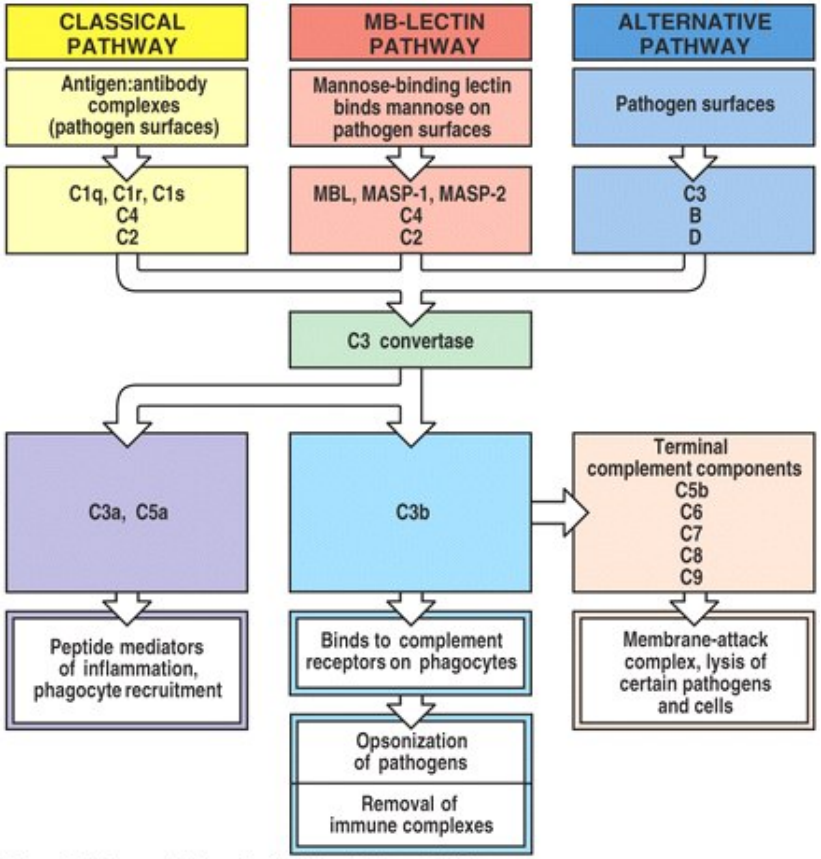


Figure 2-19 Immunobiology, 6/e. (© Garland Science 2005)

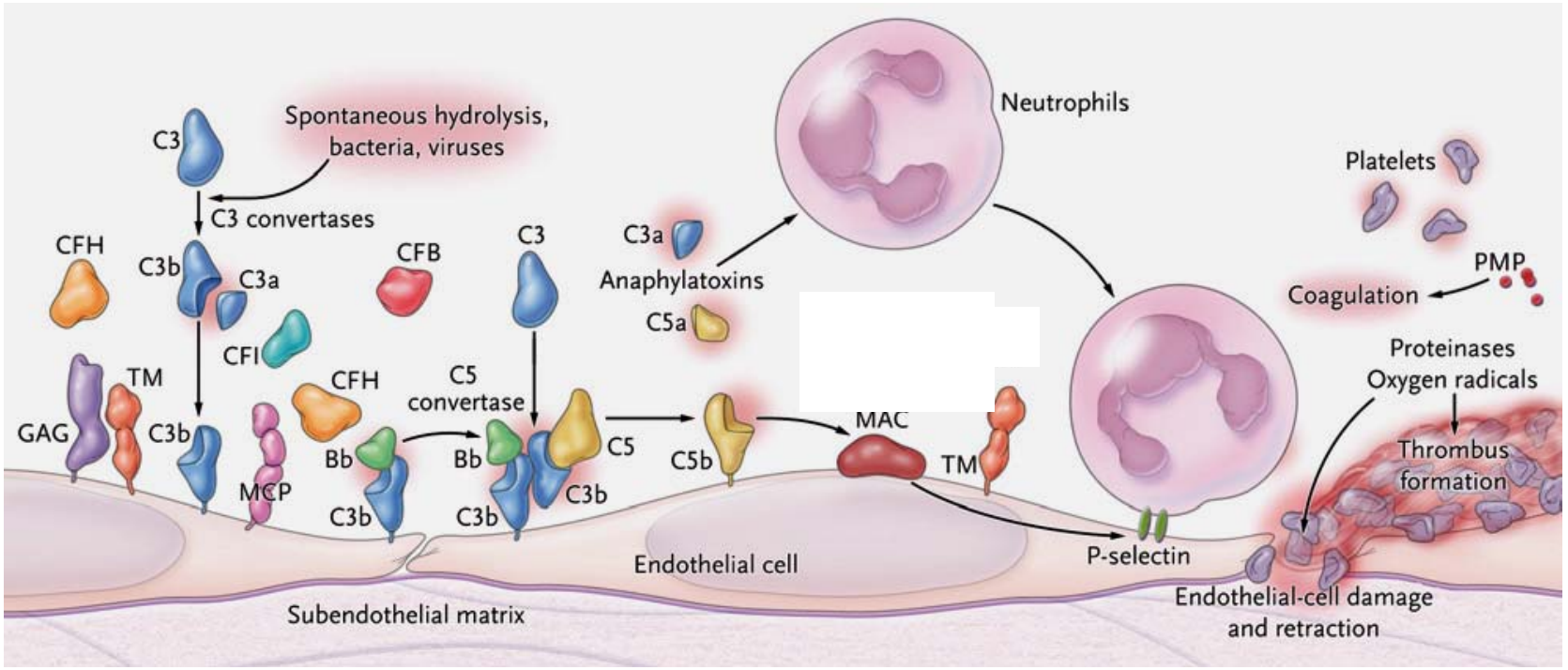
Functional protein classes in the complement system	
Binding to antigen:antibody complexes and pathogen surfaces	C1q
Binding to mannose on bacteria	MBL
Activating enzymes	C1r C1s C2b Bb D MASP-1 MASP-2
Membrane-binding proteins and opsonins	C4b C3b
Peptide mediators of inflammation	C5a C3a C4a

Figure 2-20 Immunobiology, 6/e. (© Garland Science 2005)

Functional protein classes in the complement system	
Membrane-attack proteins	C5b C6 C7 C8 C9
Complement receptors	CR1 CR2 CR3 CR4 C1qR
Complement-regulatory proteins	C1INH C4bp CR1 MCP DAF H I P CD59

Janeway, C, et al, Immunobiology, New York: Garland Science, 2005.

The Alternate Complement Pathway

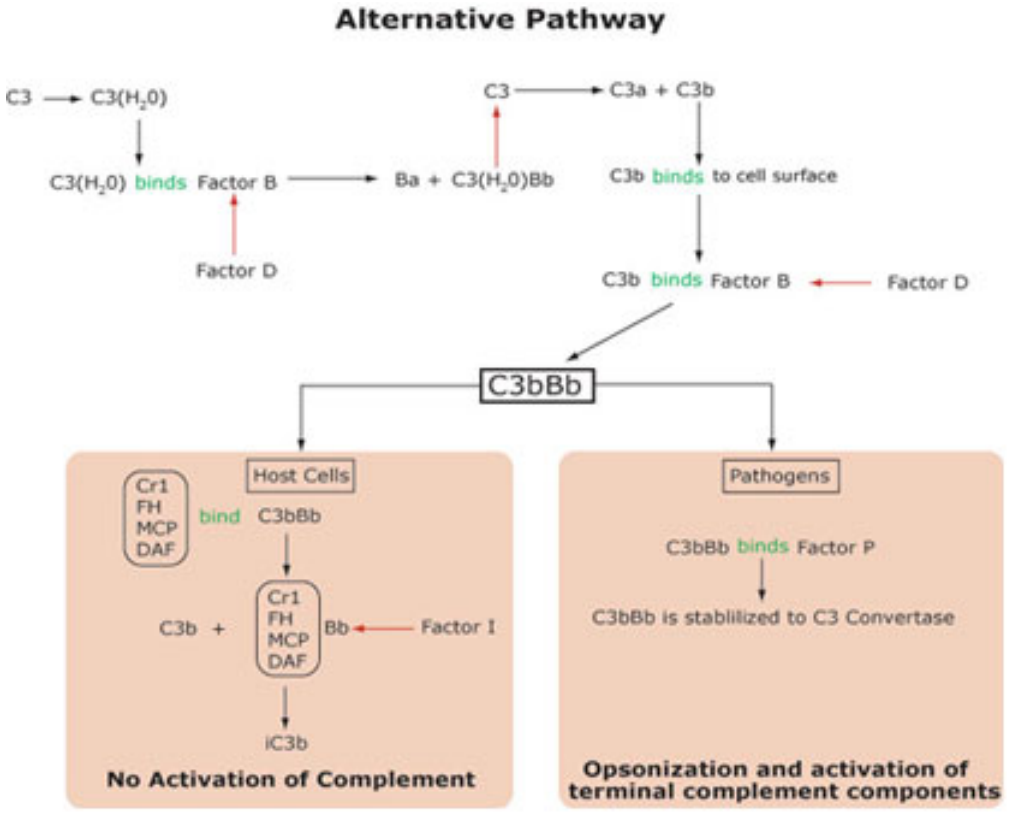


Remuzzi, NEJM, 2009 361;17

Atypical HUS

COMPLEMENT DYSREGULATION

- complement regulation



http://www.biochem.ucl.ac.uk/~becky/FH/proteinInfo.php?protein=FH

Atypical HUS

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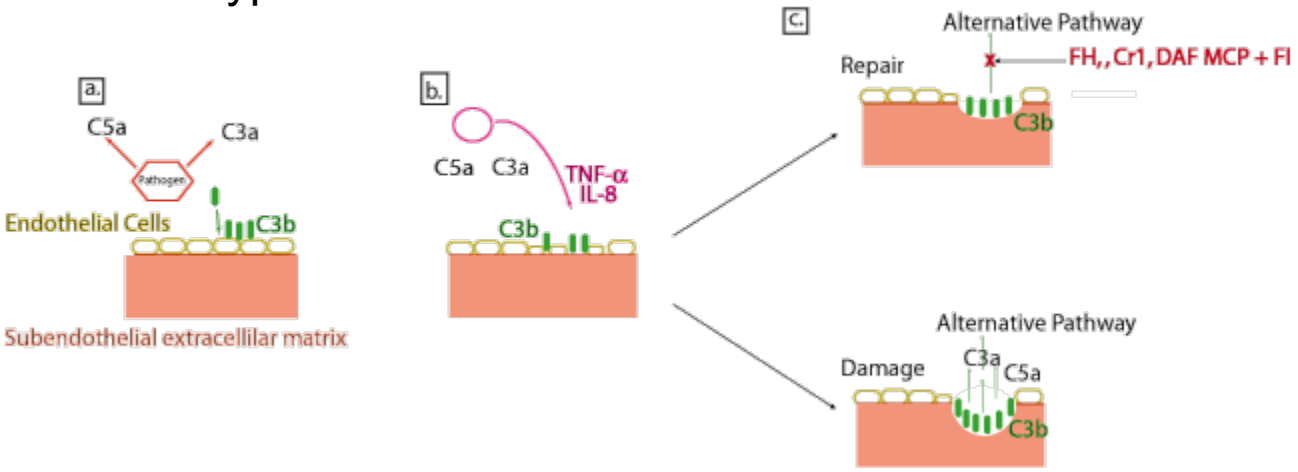
COMPLEMENT DYSREGULATION

- pathogenesis of atypical HUS



- infection/inflammation increases rate of C3b formation
- activates complement cascade and C3a/C5a
- C3a/C5a attract leukocytes, producing TNF and IL-8
- cytokines cause endothelial damage and exposure of extracellular matrix
- ECM exposure amplifies deposition of C3b and complement activation
- lack of normal factor H, factor I, or MCP results in unchecked activation
- progressive tissue damage occurs

http://www.biochem.ucl.ac.uk/~becky/FH/hus.php

Atypical HUS

COMPLEMENT DYSREGULATION

- FH, FI, and MCP deficiency have incomplete penetrance
  - disease modifiers or other factors may have role

- environmental triggers
  - infections
    - preceded 70% of those with FH mutation
    - 60% of those with FI mutation
    - 100% of cases of HUS in MCP-mutants
  - pregnancy
    - trigger in 4% of FH-HUS
    - 40% of FI-HUS

- multiple-hits
  - one pedigree in which atypical HUS occurred only with inheritance of ALL:
    - MCP P131S mutation
    - MCP promoter polymorphism
    - dinucleotide insertion into FI gene
    - resulted in 50% expression level of each protein

Richards, A, 2007, Mol Immunol 44:111-122.

Atypical HUS

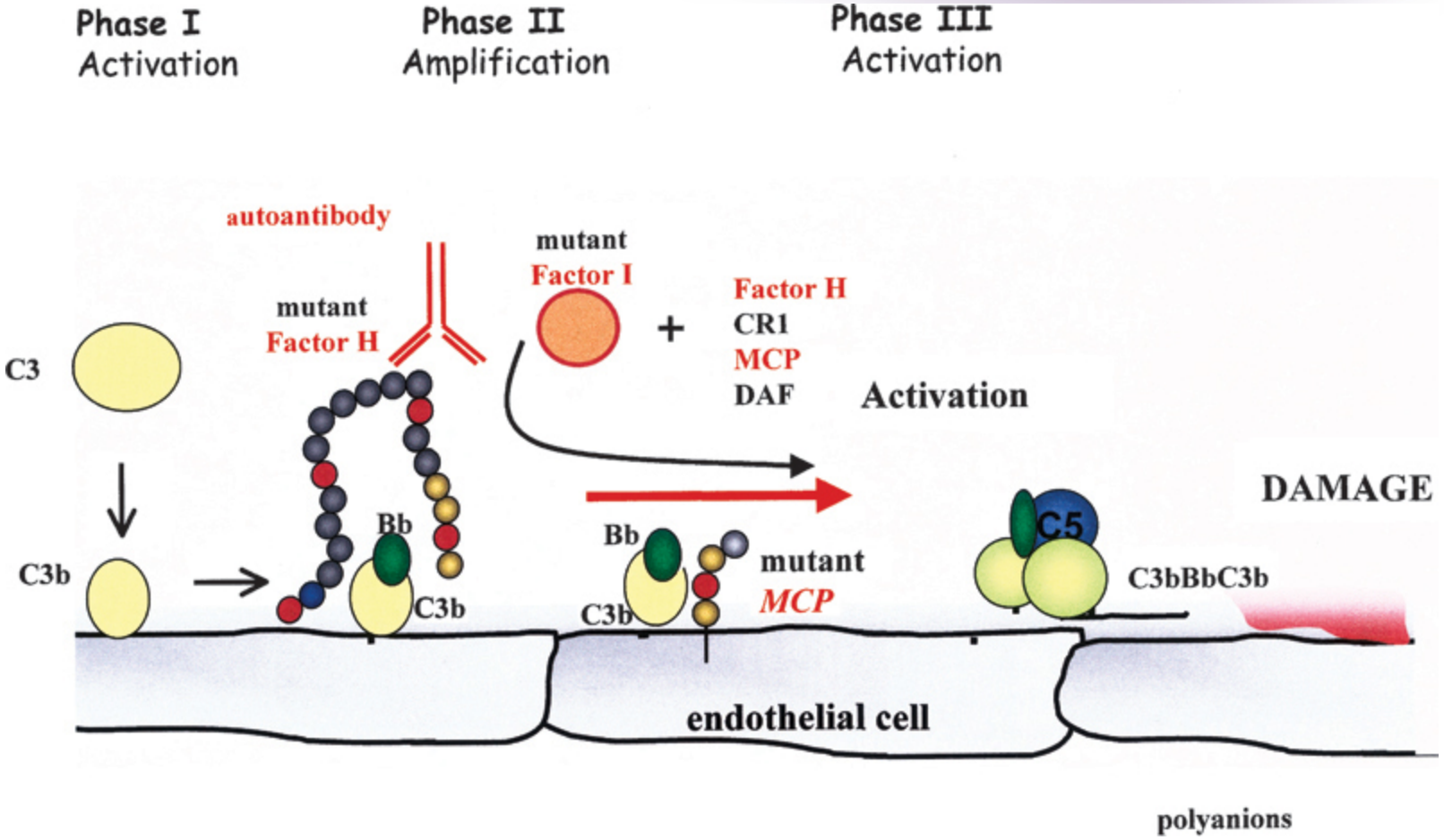
COMPLEMENT DYSREGULATION

- outcomes of atypical HUS
  - overall 50% of patients develop ESRD
  - 25% mortality during acute illness

- end-stage renal disease
  - 70% with FH-deficiency HUS develop ESRD or die
  - >60% with FI-deficiency HUS develop ESRD
  - 86% with MCP-deficiency HUS remain dialysis-free
    - 70% had recurrence of HUS

Richards, A, 2007, *Mol Immunol* 44:111-122.

SUMMARY



Zipfel, PF, et al, 2006, *Semin Thromb Hemost* 32:146-154.

Genetic Abnormalities and Clinical Outcome in Patients with Atypical Hemolytic–Uremic Syndrome

Gene	Protein Affected	Main Effect	Frequency
CFH	Factor H	No binding to endothelium	20-30%
CFHR1/3	Factor HR1, R3	Anti-factor H Antibodies	6%
MCP	Membrane cofactor protein	No surface expression	10-15%
CFI	Factor I	Low level or low cofactor activity	4-10%
CFB	Factor B	C3 Convertase stabilization	1-2%
C3	Complement C3	Resistance to C3b inactivation	5-10%
THBD	Thrombomodulin	Reduced C3b inactivation	5%

Adapted from Remuzzi, NEJM, 2009 361;17

Atypical HUS

COMPLEMENT DYSREGULATION

- factor H, factor I, or MCP deficiency accounts for 50% of atypical HUS

FACTOR H

- 150kD plasma glycoprotein synthesized in liver
- 20 homologous units of 61 residues (short consensus repeats – SCRs)

- N-terminal domains SCR1 – SCR4 bind C3b
  - complement decay accelerating activity located here
- H = three heparin binding sites
  - tertiary structure through to be bent backwards
  - exposes C-terminal SCR20

- functions as co-factor for factor I-mediated degradation of C3b,Bb

http://www.biochem.ucl.ac.uk/~becky/FH/proteinInfo.php?protein=FH

Atypical HUS

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FACTOR H DEFICIENCY

- thought to account for 10-22% of atypical HUS cases
- reported in both familial and sporadic forms
- usually presents in infancy or early childhood, but may present in adulthood

- one study of 16 FH-deficient patients
  - 6 with homozygous deficiency
    - 4 had membranoproliferative glomerulonephritis
    - 2 had atypical HUS
  - 10 had heterozygous deficiency
    - all developed atypical HUS
- homozygotes had low levels of FH, C3, FB and CH_{50}
- heterozygotes had low to normal values

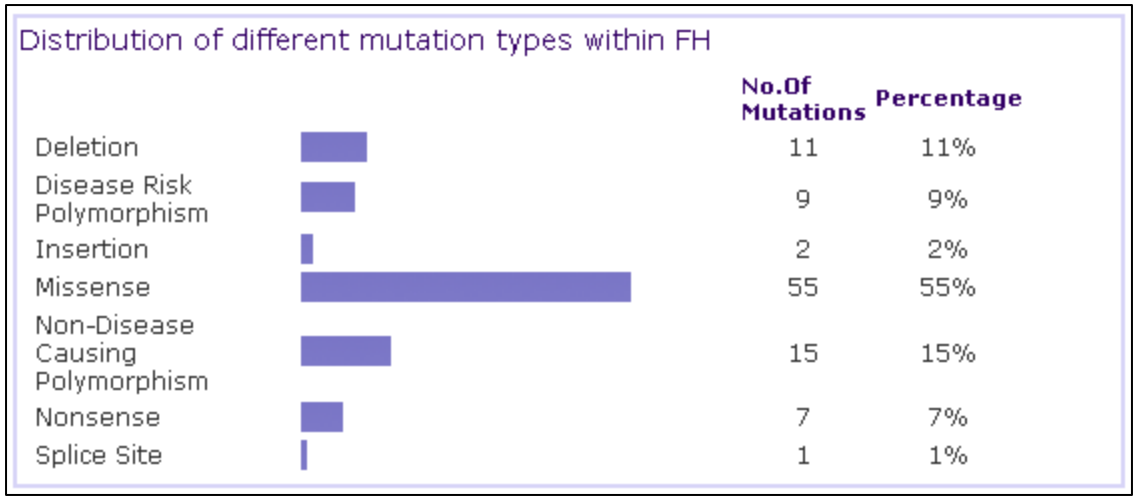
- some patients present with meningococcal infections
  - acquired C3 or terminal C' deficiencies

- some present with SLE, having combined FH and C2 deficiency

Dragon-Durey, M-A, et al, 2004, *J Am Soc Nephrol* 15:787-795.

Atypical HUS

FACTOR H DEFICIENCY



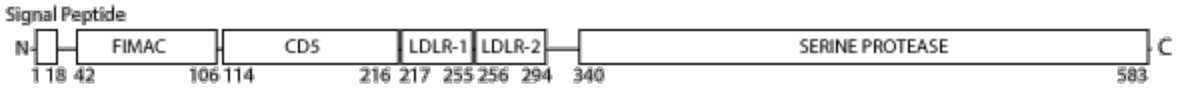
- 69 different FH mutations identified to date
- 3 patients have been described with atypical HUS and acquired anti-FH autoantibodies

http://www.biochem.ucl.ac.uk/~becky/FH//stats.php

Atypical HUS

FACTOR I

- 88kD plasma serine protease synthesized in liver



- N-terminal heavy chain
  - LDL-receptor domains x2
  - CD5 domain
  - FIMAC domain (factor I membrane attack complex)
- C-terminal catalytic domain

- functions to directly cleave C4b or C3b to inactivate complement
- efficient cleavage requires co-factors (C4bp, FH, MCP)

http://www.biochem.ucl.ac.uk/~becky/FH//proteinInfo.php?protein=FI

Atypical HUS

FACTOR I DEFICIENCY

- reported only in sporadic forms of atypical HUS
- in one study, 2 out of 76 patients with atypical HUS had FI deficiency

- most reported cases involve hyterozygous mutations
  - no increased susceptibility to infection

- homozygous FI deficiency associated with increased infection susceptibility
  - encapsulated organisms (meningococcus, pneumococcus, hemophilus)
  - acquired C3 deficiency due to uncontrolled consumption

- variable penetrance and expressivity
- C3 can be low to normal

Dragon-Durey, M-A, et al, 2005, *Springer Semin Immun* 27:359-374.
Kavanagh, D, et al, 2005, *J Am Soc Nephrol* 16:2150-2155.

Atypical HUS

FACTOR I DEFICIENCY

Distribution of different mutation types within FI

	No.Of Mutations	Percentage
Deletion	2	9%
Insertion	2	9%
Missense	7	32%
Non-Disease Causing Polymorphism	6	27%
Nonsense	3	14%
Splice Site	2	9%

- 11 different FI mutations identified to date

http://www.biochem.ucl.ac.uk/~becky/FH//stats.php

Atypical HUS

MEMBRANE COFACTOR PROTEIN (MCP = CD46)

- ~65 kD transmembrane glycoprotein
- on leukocytes, platelets, endothelial & epithelial cells, fibroblasts, kidney

- extracellular domain
  - four SCR domains
  - alternative splice sites for O-glycosylation
  - multiple isoforms exist
- transmembrane domain
- cytoplasmic C-terminal anchor

- functions as cofactor for FI
- pathogen receptor for measles, adenovirus, HHV-6, Neisseria, and GAS

http://www.biochem.ucl.ac.uk/~becky/FH//proteinInfo.php?protein=MCP

Atypical HUS

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MCP DEFICIENCY

- reported only in familial forms of atypical HUS
- both homozygous and heterozygous types seen
- 80% of patients are heterozygotes

Patient	Gender	S/F	Age at Onset of Disease (yr)	Follow-Up (yr)	No. of Relapses	Renal Involvement (yr after the onset)	Comments
Homozygous							
1	F	S	27	4	2	CRF (CrCl 34 ml/min) (4)	Pierre-Robin syndrome, common variable immunodeficiency
2	M	S	5	35	10	HD (35)	
3	F	S	2	24	2	Proteinuria 2.66 g/24 h (23)	
Heterozygous							
4	M	S	10	9	1	HD (2)	Membranous glomerulopathy with proteinuria and HT at 6; transplant at 15; graft loss at 19
5	F	S	23	0.3	0	HD[b]	Onset 3 mo after second childbirth
6	F	S	0.7	11	0	HD[b]	Three transplants with early graft loss at 4, 6, and 10
7	F	S	5	8	4	None (8)	
8[c]	F	F	3	18	4	HD (13)	Transplant at 17; lost at 21
9[c]	F	F	5	13	6	CRF (ND)	Data on clinical progression are not available
Compound heterozygous							
10[c]	M	F	1.5	3	4	None (3)	Initial presentation after *E. coli* O157:H7 infection
11[c]	F	F	8	2	0	None (2)	

Fremeaux-Bacchi, V, et al, 2006, *J Am Soc Nephrol* 17:2017-2025.

Atypical HUS

MCP DEFICIENCY

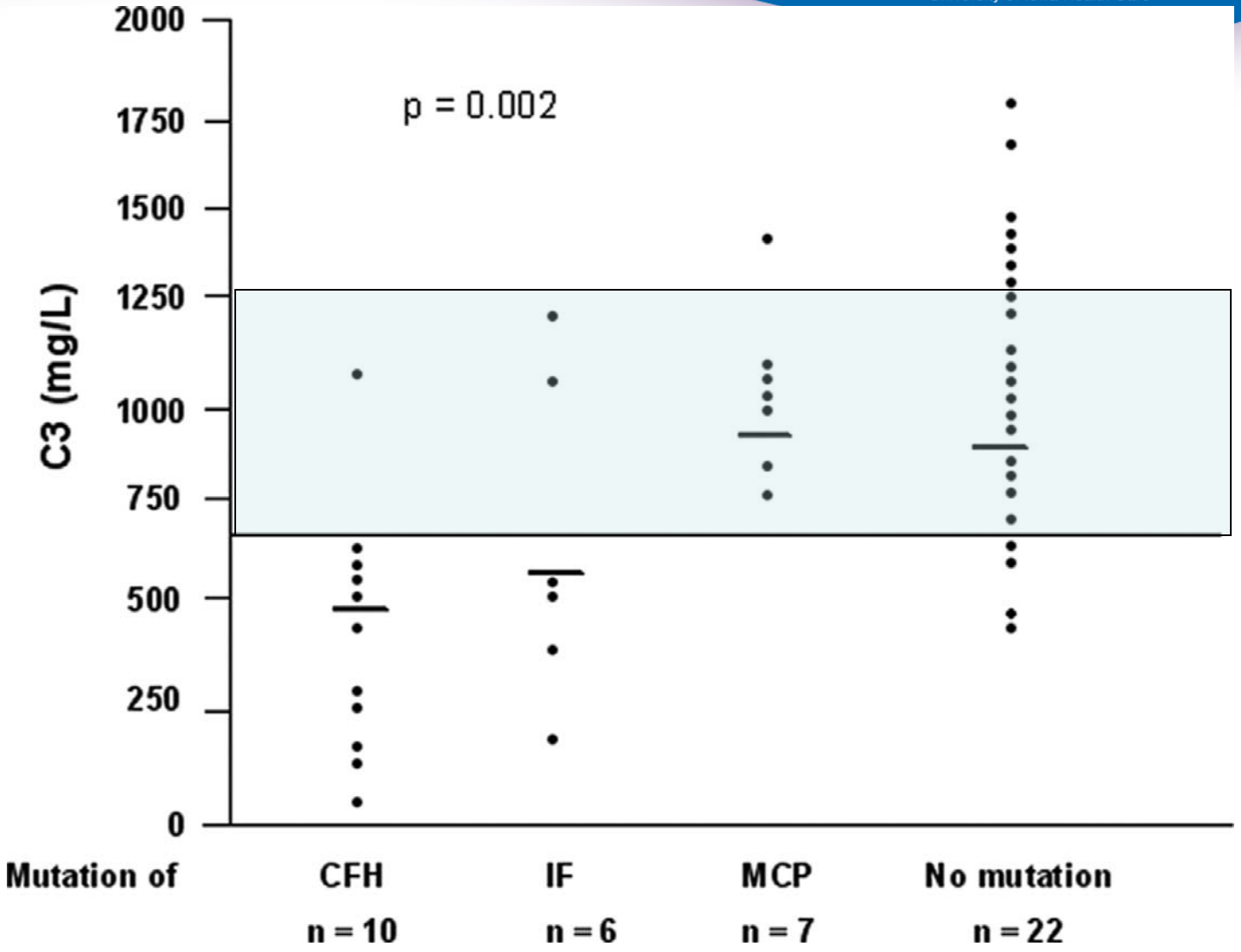
Distribution of different mutation types within MCP

	No.Of Mutations	Percentage
Deletion	6	18%
Disease Risk Polymorphism	5	15%
Missense	11	32%
Non-Disease Causing Polymorphism	4	12%
Nonsense	2	6%
Splice Site	6	18%

- 25 different MCP mutations identified to date

http://www.biochem.ucl.ac.uk/~becky/FH//stats.php

C3 Levels By Mutation



Sellier-Leclerc, A.-L. et al. J Am Soc Nephrol 2007;18:2392-2400

Complement and Atypical HUS

About 50%-60% of aHUS cases are <u>associated</u> with a mutation in a complement-related gene

Protein	Gene	Source	Location	% of aHUS
Factor H	*CFH*	Liver	circulates	~ 15-30%
Factor I	*CFI*	Liver	circulates	~ 5-10%
Membrane Cofactor Protein	*MCP*	Widespread	Membrane bound	~ 10-15%
Factor B	*CFB*	Liver, ?	circulates	<5%
C3	*C3*	Liver, ?	circulates	~ 5-10%
Anti-FH-Ab	*CFHR1/ CFHR3*	Lymphocyte	circulates	~ 10%
Unknown				~ 40-50%

Jozsi et al. Blood 2008, Frémeaux-Bacchi V et al. Blood 2008, Goicoechea de Jorge 2007, Caprioli, et al Blood 2006, Kavanagh Curr Opin Nephrol Hypertens, 2007

Recommended Initial Evaluation of HUS

Because infections trigger both typical and atypical HUS, initial evaluation should encompass both

Testing should include C3 level as well as classic evaluation (stool culture, LDH, smear, etc.)

ADAMSTS13 / auto-Ab analysis if TTP not ruled out

Save some plasma for later analysis

aHUS CONTROVERSIES

Differentiating between aHUS, TTP & STEC HUS

- Thrombocytopenia
  - Platelet count <150,000 *Or* >25% Decrease from baseline
- Microangiopathic Hemolysis
  - Schistocytes *and/or* Elevated LDH *and/or* Decreased Haptoglobin *and/or* Decreased Hemoglobin
- Neurological Symptoms
  - Confusion/Seizures/Other abnormalities
- Renal Impairment
  - Increased creatinine/decreased GFR/Abnormal UA
- Gastrointestinal Symptoms
  - Diarrhea (+/-blood), N/V, gastroenteritis, Abdominal pain

aHUS Cases

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- 3 Cases- demonstrating the issues surrounding diagnosis and utilization of clinical criteria
- Case 1- 45 yr old woman: Initially diagnosed with TTP but accurate diagnosis is aHUS
- Case 2 – 10 yr old boy: Initially diagnosed with aHUS but accurate diagnosis was TTP
- Case 3 – 12 yr old girl undergoing kidney transplant as per UI protocol

Focus on clinical presentation and accurate diagnosis

Case 1

- 45 yo Admit TMA management with atypical features (normal range platelet count). History non-bloody diarrhea. Only Medication OCP.
- Initial Diagnosis TTP
- Initially presented with foot and ankle swelling and intermittent chest tightness. Lower extremity edema since one month prior (found to have a creatinine of 3.0 and an elevated blood pressure)

Case 1

- Systemic symptoms progressed to nausea, vomiting, occasional low-grade fever and chills. Several days later followup noted to have an creatinine of 7.7, hemoglobin 7.2, and platelet count 227.
- Biopsy found thrombotic microangiopathy> Complement not done
- **Differential DIAGNOSIS: TTP vs. aHUS**
  - ADAMTS13 Activity levels at 58% (Post PEX) (N >67%). APL normal. ANA reported as abnormal. Hep screen negative. Haptoglobin low. Shistocytes 1-2+
  - Limitation of ADAMTS13 measured post PEX

its critical to utilize ADAMTS13 activity to differentiate between aHUS and TTP

Case 1

- **Six PEX Treatments:** Fluid replacement: 500 mL normal saline, 500 mL of 5% albumin, 3000 mL of plasma
- PEX initiated because of **TTP diagnosis**
- **Continued Symptoms- developed irreversible renal failure now on Chronic Hemodialysis**
- **Subsequent follow up: complement levels obtained and…**

Case 1 Labs

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	HgB		Platelets	Hapto	LDH	C3
Day						
1	8		240	20	475	
2	8.4		258			
3	8.1		224	22	435	
30						55
40						58
50						68

PEX initiated at day 1…. ADAMTS level obtained after 1st treatment Level 58% (N >67%)

Genetic Abnormalities and Clinical Outcome in Patients with Atypical Hemolytic–Uremic Syndrome: Need to assess Complement in patients with TMA

Gene	Protein Affected	Main Effect	Frequency	Response to Plasma Therapy
CFH	Factor H	No binding to endothelium	20-30%	60% Remission
CFHR1/3	Factor HR1, R3	Anti-factor H Antibodies	6%	70-80% Remission
MCP	Membrane cofactor protein	No surface expression	10-15%	No definitive indication for Therapy
CFI	Factor I	Low level or low cofactor activity	4-10%	30-40% Remission
CFB	Factor B	C3 Convertase stabilization	1-2%	30% Remission
C3	Complement C3	Resistance to C3b inactivation	5-10%	40-50% Remission
THBD	Thrombomodulin	Reduced C3b inactivation	5%	60% Remission

Adapted from Remuzzi, NEJM, 2009 361;17

Case 2

- 10 yr old male- mild fever, nausea, vomiting and some confusion (negative past history)
- HgB 7 mg/dl, plt 100, LDH 4715, Haptoglobin low, 8% schistocytes, Acute kidney injury creat- 1.4 mg/dl
- Patient referred as an aHUS patient and ADAMTS13 activity level used to differentiate from TTP
- Complement studies- initially normal
- ADAMTS 13 activity level sent-given the variable presentation of aHUS

Table 1.

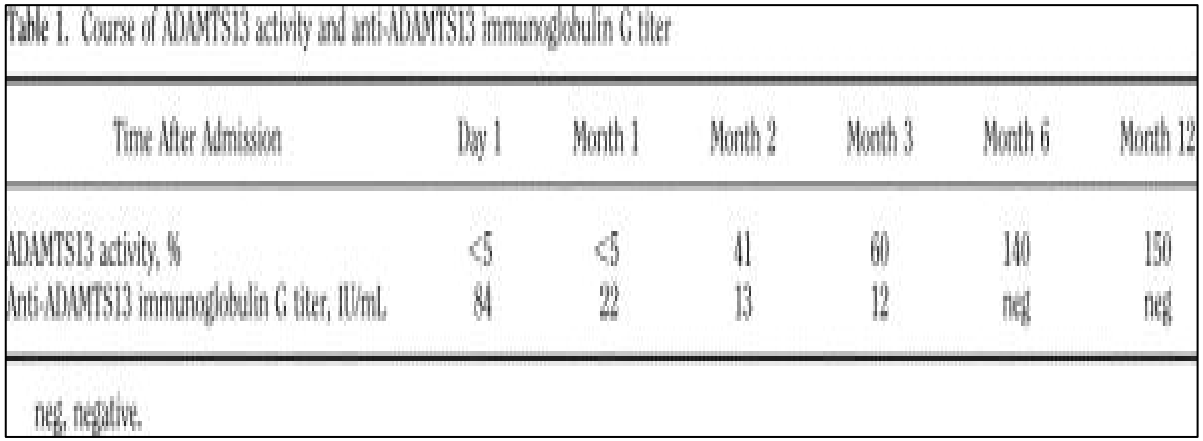


Table 1. Course of ADAMTS13 activity and anti-ADAMTS13 immunoglobulin G titer

Time After Admission	Day 1	Month 1	Month 2	Month 3	Month 6	Month 12
ADAMTS13 activity, %	<5	<5	41	60	140	150
Anti-ADAMTS13 immunoglobulin G titer, IU/mL	84	22	13	12	neg	neg

neg, negative.

Table 1. Course of ADAMTS13 activity and anti-ADAMTS13 immunoglobulin G titer

Successful treatment with rituximab for acute refractory thrombotic thrombocytopenic purpura related to acquired ADAMTS13 deficiency: A pediatric report and literature review.
Harambat, Jerome; Lamireau, Delphine; Delmas, Yahsou; Ryman, Anne; Llanas, Brigitte; Brissaud, Olivier; MD, PhD

Pediatric Critical Care Medicine. 12(2):e90-e93, March 2011.
DOI: 10.1097/PCC.0b013e3181e89f8f

ADAMTS13 to differentiate between aHUS and TTP

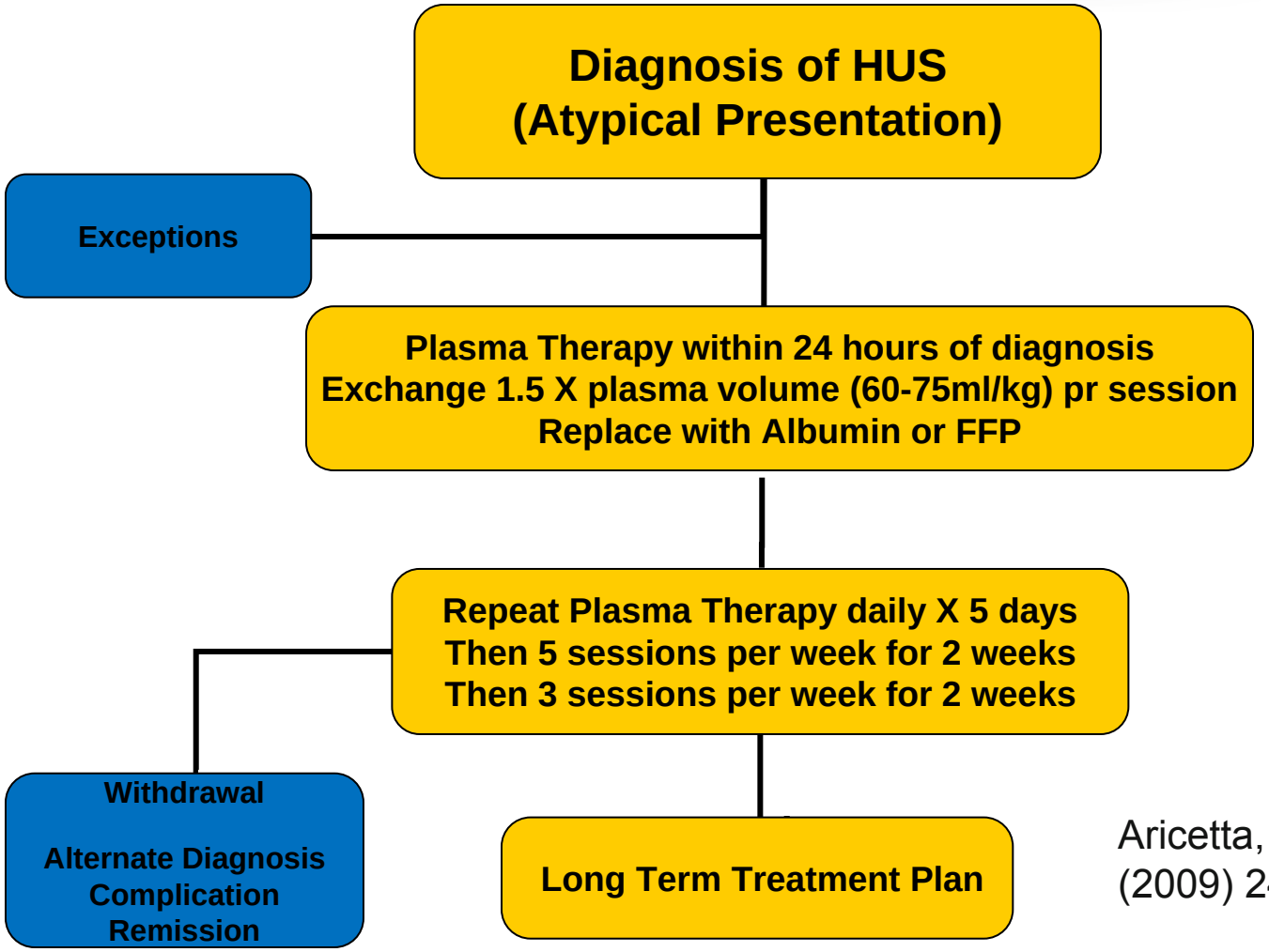
Case 2

- PEX Initiated with FFP replacement– no initial response of hematological parameters
- Condition worsened and patient developed TAMOF
- Based on ADAMTS 13 levels (<5%) and presence of strong inhibitory antibody patient started on immunosuppression
- Good Response- clinical parameters began to improve: platelets, LDH etc

Case 3

- Teenage patient- second transplant, initial diagnosis missed
- 28 Plasma Exchanges
  - Platelet 144,000 mm3 to 337,000 mm3 after thirteen Exchanges over 5 weeks
  - Hgb 5.5g/dl to 11.1 g/dl
  - Renal Function did not recover
    - Started on Chronic HD……what else?

Plasma Therapy- standard approach

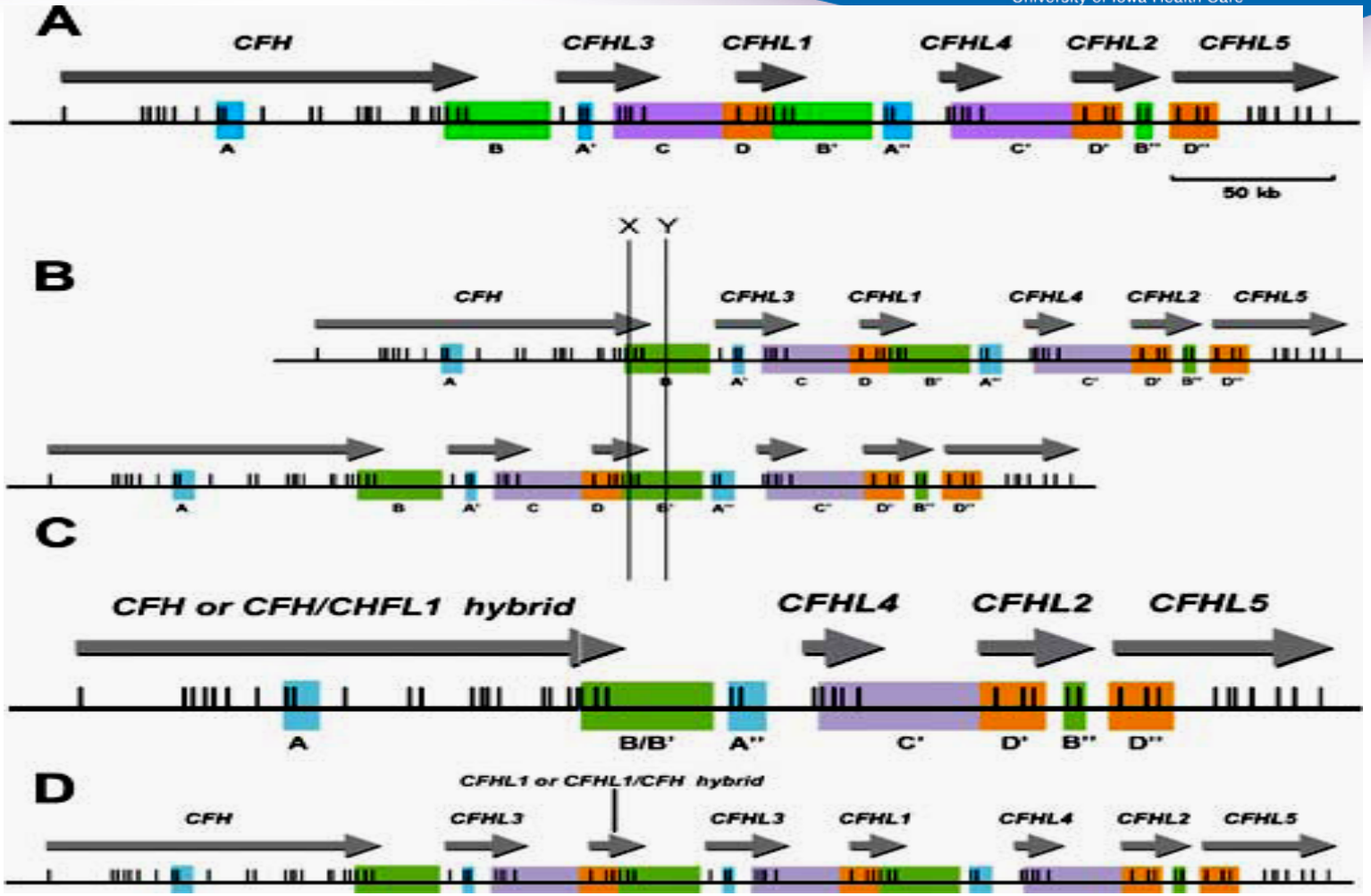


Aricetta, Ped Neph, (2009) 24:687–696

Case 3

- No mutations in *CFH*, *CFI*, *CFHR5*, *MCP*, *CFB*, *C3* or *THBD*
- only 30-50% aHUS patients have an identifiable mutation
- Copy Number studies (MPLA)
  - Hybrid *CFH/CFHR1* gene
    - Fusion protein comprised of the first 18 short consensus repeats (SCRs) of CFH and the last two SCRs of *CFHR1*

To be continued……



Venables, 2006, PLoS 3: 1957

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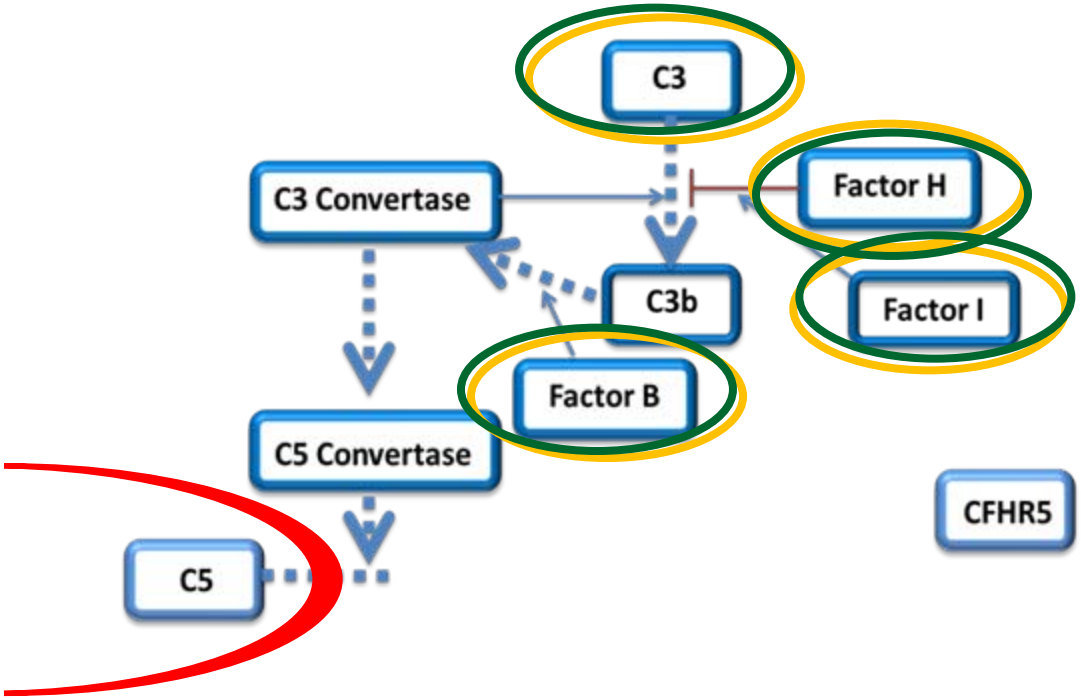
Adapted from Remuzzi, NEJM, 2009 361;17

aHUS

TREATMENT

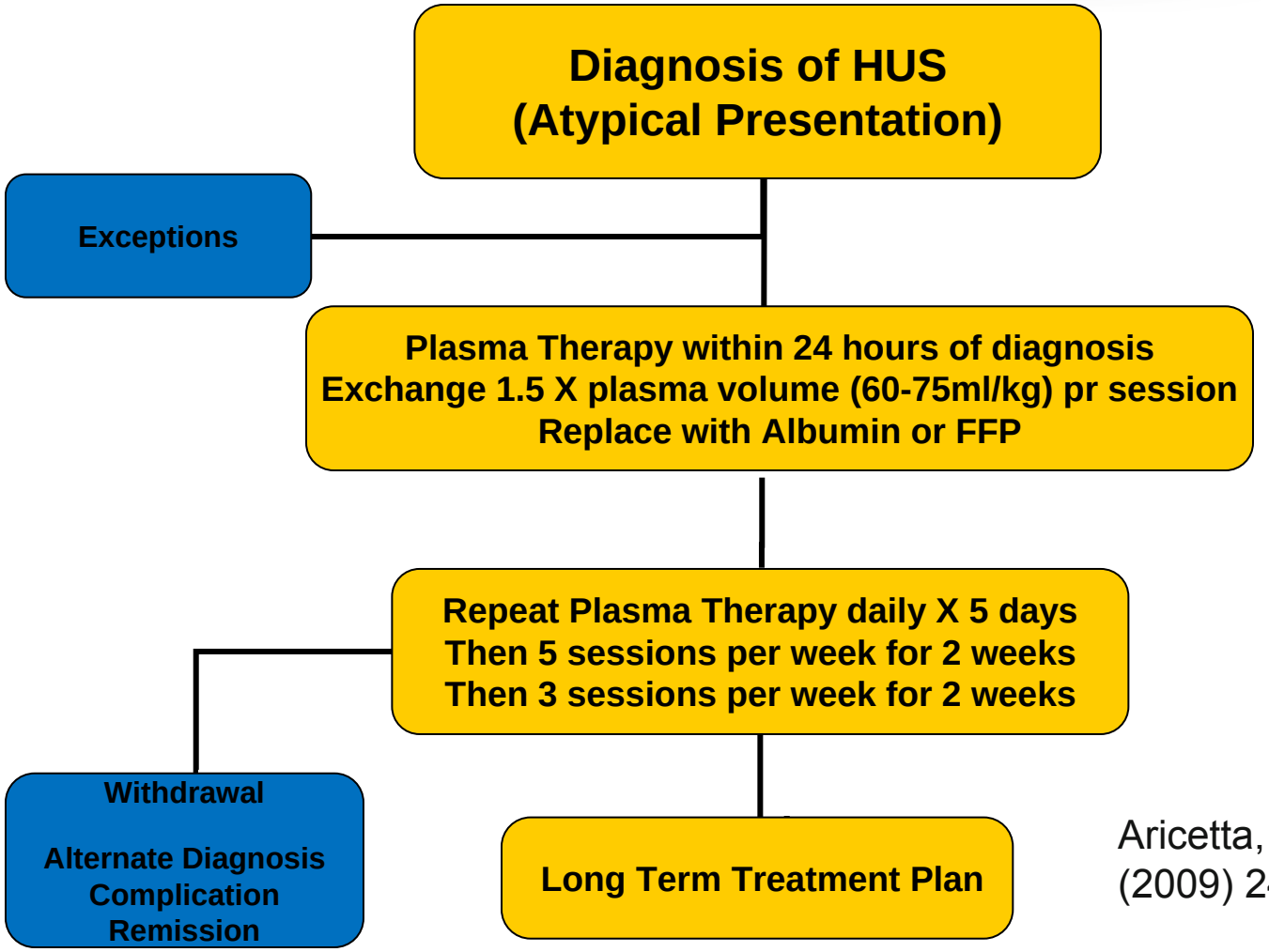
The Genetics of Atypical Hemolytic Uremic Syndrome

		European Cohort[1]			US Cohort[2]
Gene	**Role of Mutation**	**Frequency**	**Risk of ESRD at 3 years**	**Risk of transplant loss within 1 year**	**Frequency**
CFH	Mutation results in a quantitative deficiency of protein or altered binding to C3b*	23%	**77%**	**71%**	27%
MCP	Mutant proteins have low C3b binding capacity and therefore decreased cofactor activity	7%	**6%**	**0%**	5%
CFI	Mutations induce a default of secretion of the protein or disrupt its cofactor activity altering degradation of C3b/C4b	4%	**60%**	**67%**	8%
C3	Mutations interfere with binding of C3 to MCP and regulation by MCP or increased binding to CFB resulting in increased C3 convertase formation.	8%	**67%**	**43%**	2%
CFB	Mutated proteins binds excessively to C3b and stabilizing the C3 convertase making it resistant to decay by CFH, enhancing formation of C5b-9 complexes and deposition of C3-fragments onto endothelial cell surfaces	1%	-	-	4%
THBD	Mutated proteins are less effective at moderating CFI-mediated inactivation of C3b	5%	**54%**	**0%**	3%
CFHR1/3	Associated with CFH Antibodies	6%	-		
CFHR5	Unknown	Not Reported			3%
Fusion Proteins	Results in non-functional CFH	Not Reported			Not Reported
CFH Antibody	Anti-CFH IgG bind to CFH and inhibit CFH binding to C3b and cell surfaces	3%	**63%**		Not Reported
Unknown		52%	**50%**		54%



1. Remove abnormal proteins
2. Replace deficient proteins
3. Block terminal complement effects.

Plasma Therapy- standard approach



Aricetta, Ped Neph, (2009) 24:687–696

Plasmatherapy

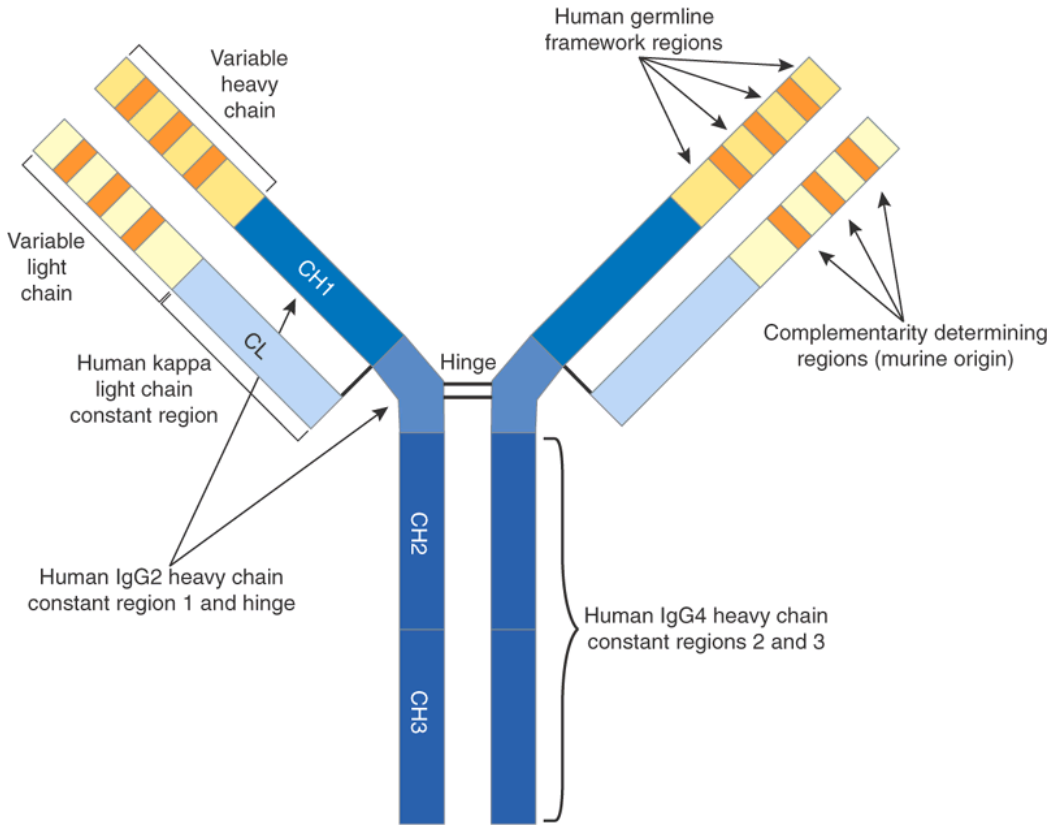
CFH Mutation	63% Hematologic Response
CFI	25% Hematologic Response
C3	57% Hematologic Response
Thrombomodulin	88% Hematologic Response

CFH Mutation	63% Hematologic Response
CFI	25% Hematologic Response
C3	57% Hematologic Response
Thrombomodulin	88% Hematologic Response

CFH Mutation	37%	Death or ESRD
CFI	75%	Death or ESRD
C3	43%	Death or ESRD
Thrombomodulin	13%	Death or ESRD

CJASN, 2010;5:1844-59.

Eculizumab



- Recombinant, humanized, monoclonal antibody directed against C5 – specifically preventing its cleavage by the C5 convertase.
- Prevents the generation of the terminal complement complex C5b-9.
- The single most expensive drug in the world. ($409,500/yr – *Forbes Magazine*)

Nature Biotechnology 25, 1256 - 1264 (2007)

Eculizumab and aHUS

N Engl J Med. 2009 Jan 29;360(5):542-4

N Engl J Med. 2009 May 14;360(20):2142-3

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Am J Kidney Dis. 2010 Apr;55(4):708-11.

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Clin J Am Soc Nephrol. 2011 Jun;6(6):1488-94.

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Pediatr Nephrol. 2012 Aug 19.

Pediatr Nephrol. 2012 Sep 6.

Am J Transplant. 2012 Sep 7.

Terminal Complement Blockade

Eculizumab and Atypical Hemolytic Uremic Syndrome		
Patients Resistant to Plasma Therapy - 26 Week Results		
Change in Platelet Count	+ 96 ± 21 X 10^9	P = <.0001
TMA Event Free Status	15/17 patients (88%)	95% CI 64-100
Change in Renal Function		
One stage improvement	11/17 patients (65%)	
Less than one stage	4/17 patients (23%)	
Removal from dialysis	5/7 patients (71%)	
Quality of Life Improvement	EuroQol 5D: 0.33 ± 0.09	P = <0001
Patients on Chronic Plasma Therapy - 12 week Results		
TMA Event Free Status	13/15 patients (87%)	95% CI 60-98
TMA Interventions	0/17 patients (0%)	
Pediatric Patients Exposed to Eculizumab - Retrospective Review of 4 Week Data		
Hematologic Parameters	Normalization in 8/19 patients (42%)	
Change in Renal Function	≥ 15ml/min/1.73m^2 improvement in 7/19 patients (78%)	
Removal from dialysis	4/8 patients (50%)	

FDA Approval September 2011

Liver-Kidney Transplant- Option 1

- Goal: correct genetic defect
  - Liver protein replacement strategy
- Initial attempts - high mortality
  - Early failure of transplant liver
- 7/8 with extensive PTx have retained their liver
  - 8th died of hepatic encephalopathy

Liver Transplant

- CFH, CFI, CFB and C3
- 20 Cases (Presse Med. 2012; 41: e115–e135)
- 4 procedures in 2002 – All Fatal
- With preconditioning - 86% patient survival reported
- Protection against kidney transplant rejection
- No reports of aHUS recurrence

Table 6. Guidelines to surgery and perioperative treatment of patients receiving a combined liver and kidney transplant or a liver transplant alone[a]

Dialysis[b]
better before plasma exchange in all cases
mandatory before plasma exchange in cases with evidence of complement activation (e.g., angioedema) during dialysis
Plasma exchange[b]
a minimum of 1.5 Vol of FFP is exchanged within 4 to 6 h of surgery
exchange must be repeated if surgery is delayed
Plasma infusion
10 to 20 ml/kg body wt FFP is infused intraoperatively after native hepatic explant
additional plasma may be given as clinical need dictates
Surgery
split or whole liver transplantation is indicated
adequate liver mass must be provided (minimum 2% liver to recipient mass ratio)
auxiliary liver transplantation is not recommended
living-related donation is not recommended
Monitoring
posttransplantation liver function should be judged by coagulation profile
in cases of inadequate liver function, plasma exchange in conjunction with standard care is indicated[c]
Posttransplantation anticoagulation[d]
low molecular weight heparin at prophylactic dosages (*e.g.*, enoxaparin 0.5 mg/kg twice daily)
aspirin (2 mg/kg per d up to 80 mg/d)
to be continued for 3 mo
Immunosuppression
per standard practice of each center
mTOR inhibitors are not encouraged

Eculizumab

J Am Soc Nephrol. 2009 May;20(5):940-9

Liver Transplant

- PEX with FFP 4-6 hours before transfer to OR
- Eculizumab
- Intraoperative transplant immune suppression
- Hepatectomy → Whole-liver orthotopic transplant
- Intra-operative FFP after liver perfusion
- Kidney transplant
- Eculizumab
- Heparin/ASA

Am J Kidney Dis. 2011;58(1):109-

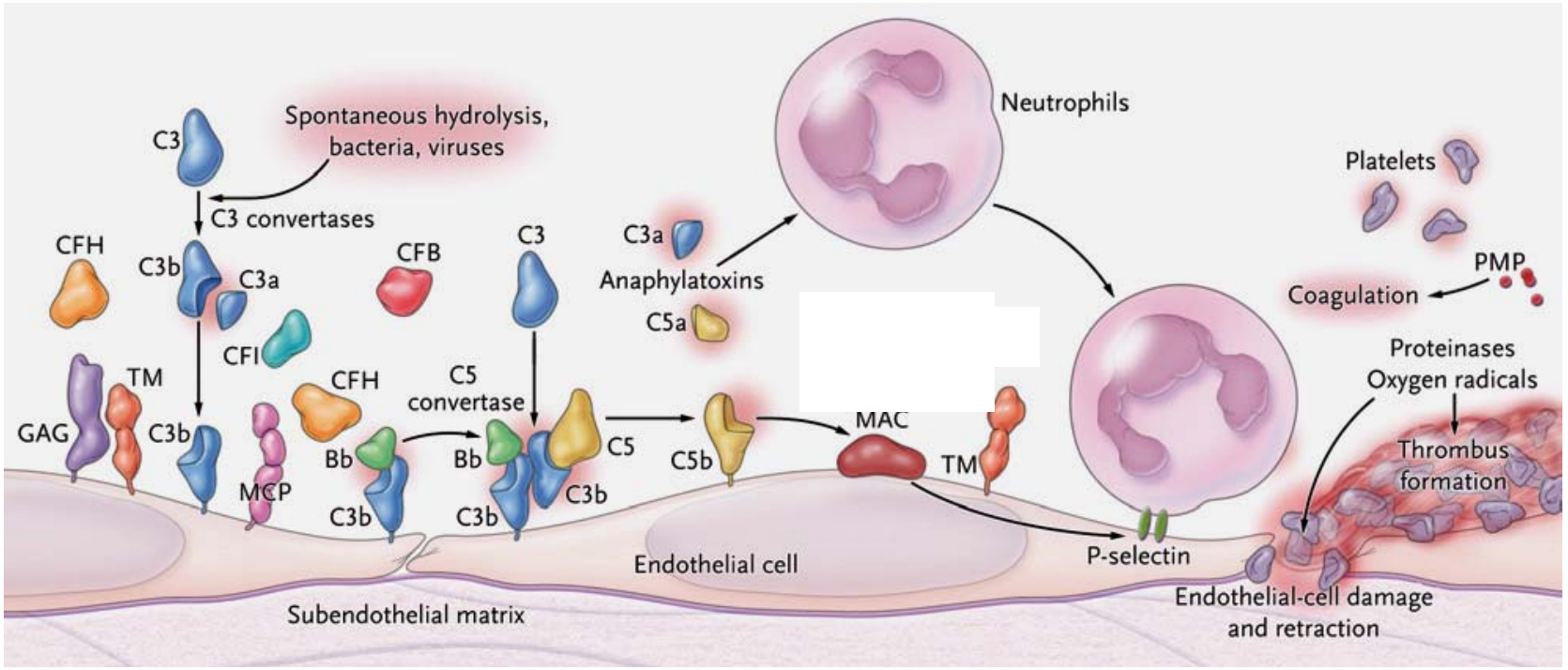
Impediments to Care

- Consideration of the diagnosis
- Availability of diagnostic studies
- Decision to PEX or Ecu acutely may be based on logistics
  - Local Expertise
  - Formulary concerns
  - Hospital financial concerns

New Kidney + Plasma Therapy & Eculizumab

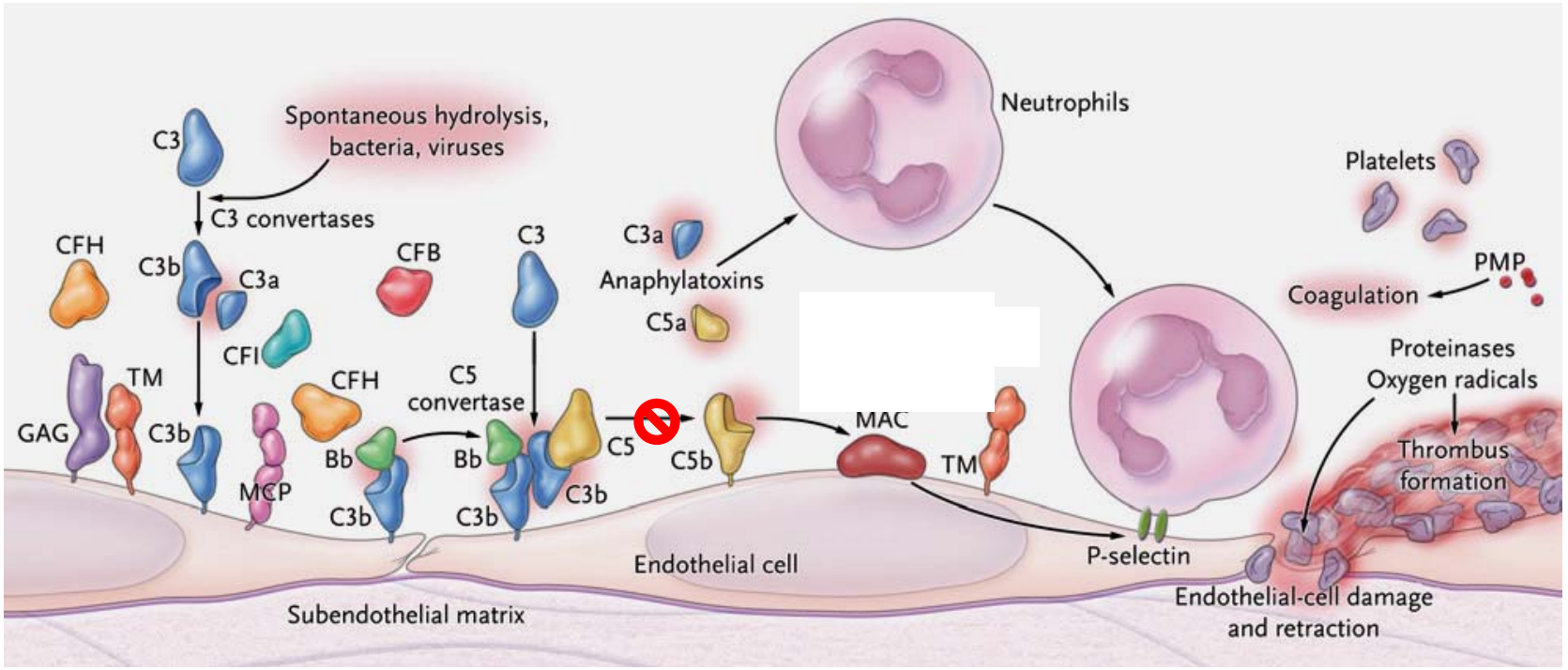
Option 2

The Alternate Complement Pathway



Remuzzi, NEJM, 2009 361;17

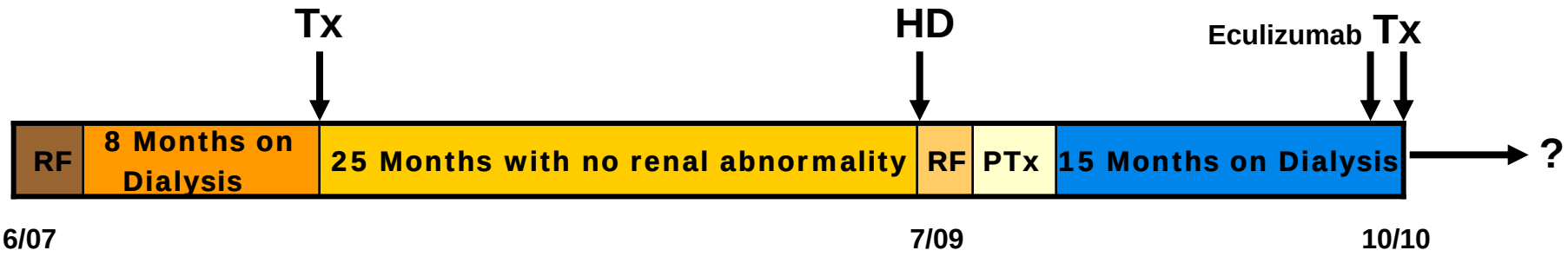
The Alternate Complement Pathway



Remuzzi, NEJM, 2009 361;17

Case 3

- On dialysis for 15 months
- Altruistic donor comes forward
- Rare Renal Disease Team approval
- Renal Transplant Protocol Approved
  - Eculizumab and Plasma Therapy
- Transplant 10/07/10



Concerns

- Genetic Testing
- Efficacy
- Life time risk of infection
- Surveillance
- Length of Therapy
- Payment
- On going research

Patient Update

- Creatinine 0.9, 16 months s/p transplant
- No bacterial infections
- Returned to school full time
- 1/13 H&H 12.2/36 → 3/10 6.3/18
  - WBC, platelets normal
  - Borderline low C3
  - What else do you want to know?

- Hemolytic Workup Negative
- Bone Marrow Biopsy
  - Mildly hypocellular bone marrow (50-70%) showing normal granulopoiesis, normal megakaryocytes and markedly decreased erythropoiesis with maturation arrest and giant normoblasts with viral inclusions
  - Now what do you want to know?

- Parvovirus B19 found in bone marrow

- Cellcept discontinued
- 1 Unit PRBC given 3/10
- 2nd Unit given 3/24

Pre-transplant Evaluation

- Donor evaluation for pathogenic aHUS mutations
- Immunize against meningococcus (as well as hemophilus and pneumococci if not current)
- Verify titer if on dialysis or if immune suppressed at the time of vaccination – consider re-immunization and antibacterial prophylaxis as necessary.

Zero minus 1 week (prior to transplant)

- Measure C3*, C4, AH50, CH50, C5 Functional Level, CBC, LDH, haptoglobin, platelet count and if appropriate C3Nef, CFI and/or CFI levels.
  Labs must be drawn before plasma therapy and before eculizumab
- Plasma exchange or Infusion – **1st dose** - (1.5 volumes of Albumin)
- Administer 900 mg eculizumab – **1st dose**

Zero minus (24-48 hours prior to transplant)

- Measure C3, C4, AH50, CH50, C5 Functional Level, CBC, LDH, haptoglobin, platelet count and if appropriate C3Nef, CFI and/or CFI levels.
  Labs must be drawn before plasma therapy and before eculizumab
- Plasma exchange or infusion – **2nd dose** – (1.5 volumes of FFP)

Zero hour (0-24 hours before transplant)

- Administer 900mg eculizumab – **2nd dose**

Post-transplant

- Measure C3, C4, AH50, CH50, C5 Functional Level, CBC, LDH, haptoglobin, platelet count and if appropriate C3Nef, CFI and/or CFI levels.
  Labs must be drawn before plasma therapy and before eculizumab
- Administer 900mg eculizumab q7 days times two (**3rd and 4th dose**)
- Administer 900mg eculizumab q 14 days

University of Iowa Children's Hospital
University of Iowa Health Care

Kidney Transplant

Univ of Iowa OTC ver 1.4 Jan 2012

Protocol for the prophylactic use of eculizumab for renal transplant in a patient with atypical HUS [1]

Pre-transplant Preparation

Donor

- Unrelated Living Donor: (Preferred option). Usual pre-donation transplant workup.
- Related Living Donor: (Less desirable option [2]). Donor should be evaluated for pathogenic aHUS mutations[#].
- Deceased Donor: (Less desirable option). Usual donor workup. Will require adjustment of the recipient protocol based on type of genetic mutation in recipient, anticipated cold ischemic time, prior history of transplant and current PRA.

Recipient

- Immunize recipient against meningococcus – meningococcal conjugate vaccine (if 2 through 55 years of age) - 2 doses 2 months apart (immunize against hemophilus and pneumococci if not currently immunized).
- Verify titer 1 month after 2nd dose if on dialysis or if immune suppressed at the time of vaccination – consider re-immunization and antibacterial prophylaxis as necessary.
- If unable to immunize at least two weeks in advance of first dose of eculizumab, must use anti-meningococcal antibiotic coverage for the first 14 days post immunization.

Zero minus 4-7 days (prior to transplant)(for living donor recipients only)

- Measure C3**, C4, AH50, CH50, C5 Functional Level, CBC, LDH, haptoglobin, platelet count and if appropriate CFH, CFI and/or autoantibody levels (C3Nef, FHAA, FBAA)
- One red top and one pink top for immediate isolation of serum and plasma for storage[@]
  Labs must be drawn before eculizumab
- Administer 900 mg eculizumab– **1st dose**
- One red top and one pink top for immediate isolation of serum and plasma for storage[@]
  Labs must be drawn 60 minutes after eculizumab

Zero minus 1 day (prior to transplant) (for all recipients)

- Measure C3**, C4, AH50, CH50, C5 Functional Level, CBC, LDH, haptoglobin, platelet count and if appropriate CFH, CFI, CFB and/or autoantibody levels (C3Nef, FHAA, FBAA)
- One red top and one pink top for immediate isolation of serum and plasma for storage[@]
  Labs must be drawn before plasma therapy and before eculizumab
- Plasmapheresis (PE) – **1st dose[$]** - (1 volume of FFP)
- Administer 1200 mg eculizumab after plasma exchange– **2nd dose for LD recipients (1st dose for DD recipients)**

Zero hour (immediately prior to OR - day of transplant for all recipients)

- Measure PT, PTT, C3, C4, AH50, CH50, C5 Functional Level, CBC, LDH, haptoglobin, platelet count and if appropriate CFB, CFI and/or autoantibody levels (C3Nef, FHAA, FBAA)
- One red top and one pink top for immediate isolation of serum and plasma for storage[@]
  Labs must be drawn 60 minutes after eculizumab

Post-transplant

Post-Op Day Zero (upon discharge from OR)

- Measure C3, C4, AH50, CH50, C5 Functional Level, CBC, LDH, haptoglobin, platelet count and if appropriate CFB, CFI and/or autoantibody levels (C3Nef, FHAA, FBAA)
  One red top and one pink top for immediate isolation of serum and plasma for storage[@]
- Administer 1200 mg eculizumab for DD recipients only (**2nd dose for DD recipients**)

Daily

- Monitor Hb, platelets, renal function, haptoglobin and LDH

Day 7, Day 14, and Day 21

- Measure C3, C4, AH50, CH50, C5 Functional Level, CBC, LDH, haptoglobin, platelet count and if appropriate CFH, CFI and/or autoantibody levels (C3Nef, FHAA, FBAA)
  Labs must be drawn before eculizumab
- Administer 900-1200 mg eculizumab on day 7, 14, and 21 (3rd, 4th and 5th dose)

Day 35 Postop

- Measure C3, C4, AH50, CH50, C5 Functional Level, CBC, LDH, haptoglobin, platelet count and if appropriate CFH, CFI and/or autoantibody levels (C3Nef, FHAA, FBAA)
  Labs must be drawn before eculizumab
- Administer 900-1200 mg eculizumab q 14 days starting with the **6th dose**.

The following measures to be considered if ongoing hemolysis is noted: Plasmapheresis with FFP or albumin. Additional doses of eculizumab may be necessary if apheresis is performed, or if breakthrough hemolysis occurs

Notes:

[#] Mutation screening of *CFH, MCP (CD46), CFI, CFB, C3, THBD* and MLPA for copy number analysis of *CFHR1* and *CFHR3* performed at the Molecular Otolaryngology & Renal Research Laboratory, Iowa City

[@]Contact Amy Weaver (335-6623), or Richard Smith (Richard-smith@uiowa.edu), MORL, Iowa City.

[$]Consider additional plasmapheresis pre-transplant if autoantibody present or if CFH mutation is expected to result in a gain of function or dominant negative effect.

Eculizumab - drug Interactions.

A number of therapeutic antibodies are used in the management of kidney transplant recipients. Antibodies, such as rituximab [3] and alemtuzumab[4], that exert its action primarily by complement dependent cytotoxicity (CDC) may be inhibited by concurrent administration of eculizumab. Thymoglobulin depletes peripheral T cells by a combination of CDC, antibody dependent cellular cytotoxicity (ADCC) and activation induced apoptosis [5]. If thymoglobulin is used with eculizumab monitor its efficacy by measurement of absolute lymphocyte or CD3 count. Basiliximab competitively inhibits IL-2 binding to activated T lymphocytes and does not require complement for its action [6].

"Abbreviations:

C3, complement component 3; C4, complement component 4; AH50, alternate complement pathway hemolytic assay; CH50, total complement pathway hemolytic assay; C5, complement component 5; CBC, complete blood count; C3Nef, complement component 3 nephritic factor; CFI, complement factor I level; CFH, complement factor H level; FHAA, factor H autoantibody; FBAA, factor B autoantibody; q, every

References

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Molecular Otolaryngology & Renal Research Laboratory

- University of Iowa
  - Dr. Richard Smith
- CLIA Testing
- Research Testing – Clinician/Patient driven
  – IRB Mechanism

http://www.healthcare.uiowa.edu/labs/morl/

MORL

(Molecular Otolaryngology & Renal Research Laboratory)

- Not for profit Lab
- Diagnostics section
  - CLIA certified
  - aHUS testing
  - screen all KNOWN genes for ANY possible variant.
  - will detect novel mutations and previously reported mutations
- Discovery section
  - Genetics of rare renal diseases including atypical HUS and Dense Deposit Disease (one section)
  - New assays in development

Specialized Testing

- Adam TS 13 mutations- ELISA
  - TTP analysis

- sMAC testing
  - only lab in US- some with DDD and all with C3 GN-
- Only ~50% DDD patients successful in transplantation those seem to have sMAC (eculizumab)

aHUS

- 260 cases processed and diagnosed in lab
- Large genetics repository- goal to develop more fully a complete registry
- Other tests – recent German cases: may have complement abnormalities underlying as well—EHEC may help ID those with underlying genetic predisposition

Characterization of an American aHUS Cohort

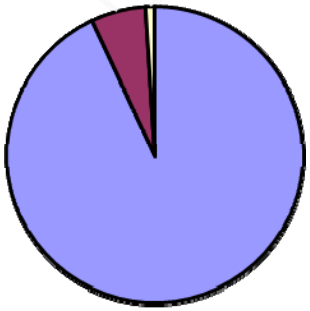


Table 1: Clinical outcomes of aHUS patients (n=70)

Clinical Outcome	Patients
Renal Failure	57* (81%)
No Relapse	13 (19%)
Renal Transplant	13 (19%)
Transplant Failures	7 (54% of all transplants)

*4 patients deceased

Table 2: Clinical outcome for each mutated protein

Mutated Protein	Renal Failure	Failed Transplant*	No Relapse	Unknown Clinical Outcome	Total
CFH	16	4	2	16	34
CFHR5	-	-	1	1	2
CFI	4	-	-	4	8
MCP	1	-	1	4	6
CFB	2	-	-	1	3
C3	-	-	-	3	3
THBD	1	-	-	1	2
CFH/CFI/MCP	1	-	-	-	1
CFH/CFHR5	-	-	-	1	1
CFH/CFB	1	-	-	1	2
CFH/CFI	1	-	-	-	1
CFI/CFB	1	1	-	-	1
THBD/CFHR5	-	-	-	1	1
THBD/CFI/CFI	1	-	-	-	1
No Mutations	28	2	9	41	78
Total	**57**	**7**	**13**	**74**	**144**

*These patients are included in the number of patients in renal failure (Column 2)

Prevalence of Mutations in American Cohort

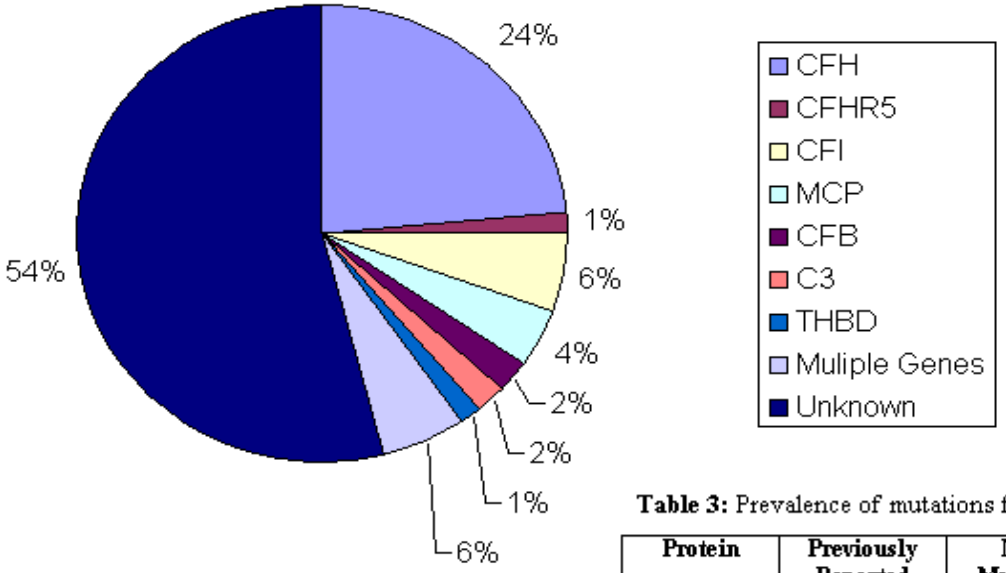
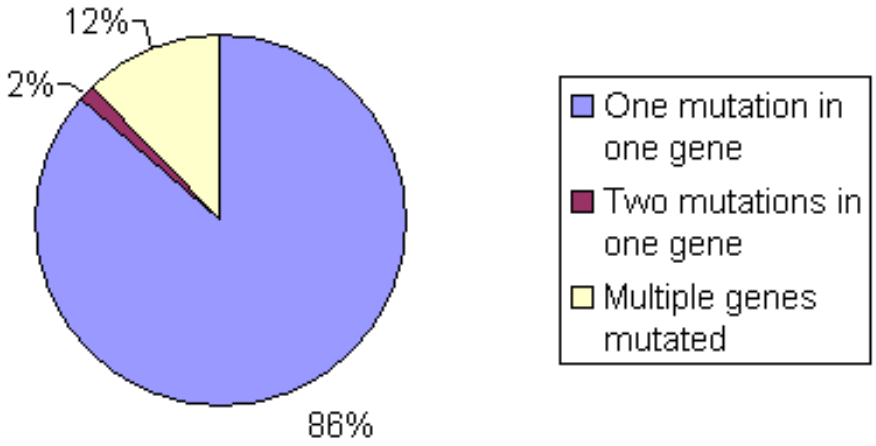


Table 3: Prevalence of mutations found in American aHUS cohort (n=144)

Protein	Previously Reported Mutations	Novel Mutations	Total Mutations	Number of Patients	Percentage in Cohort	Reported Frequencies
CFH	9	9	18	39	27%	20-30%
CFHR5	0	3	3	4	3%	-
CFI	2	7	9	12	8%	4-10%
MCP	2	5	7	7	5%	10-15%
CFB	0	6	6	6	4%	1-2%
C3	1	2	3	3	2%	5-10%
THBD	2	2	4	4	3%	5%
Total	16	34	50	66*	46%	-
Unknown	-	-	-	78	54%	-

*Correction for patients with either a mutation in more than one gene or multiple mutations in a single gene

American aHUS Patients Carrying Multiple Complement Gene Variants



Category	Number	Patient	Genes
Patients who have more than one mutation in the same gene	1	1679	*CFI* (p.G119R and p.G287R)
Patients who have mutations in more than one gene	8*	aHUS-12	*CFH, CFI, MCP*
		770	*CFH, CFB*
		1964	*CFH, CFB*
		1939	*CFH, CFI*
		aHUS-07	*CFI, CFB*
		1679	*CFI, THBD*
		511	*CFH/CFHR5*
		1525	*CFHR5/THBD*

Summary of Results

University of Iowa Children's Hospital
University of Iowa Health Care

- Percentage of Mutations found in American patients
  - Similar
    - Overall mutations found ~50%
    - CFH Mutations ~30%
    - CFI ~10%
    - THBD ~5%
  - Increased
    - CFB ~4% compared to ~2% (doubled)
  - Decreased
    - CD46 ~5% compared to ~10% - Likely due to the level of severity in our cohort (half)
    - C3 ~2% compared to ~10% (one quarter)
- Important to screen all known susceptibility genes
  - 12% of patients that were mutation positive carried more then one mutation
- Likely that rare variants are important in disease
- Thrombomodulin- unsure of the significance of the variants at this point
- Ethnic controls are important

University of Iowa
Rare Renal Disease Clinic

- Research: Dr Smith and his research team
- http://www.healthcare.uiowa.edu/labs/morl/

- Clinical- held in the Pediatric Specialty Clinics
- Dr. Richard Smith
- Dr. Carla Nester
- Dr. Christie Thomas
- Dr. Alan Reed
- Dr. Pat Brophy
- Monica-keleher@uiowa.edu

Thank You

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- Jeff Saland
- Gary Pien
- Brad Dixon

- Rare Renal team
  University of Iowa